



## Modeling green microalgal growth, nutrient uptake and storage in the ASM framework

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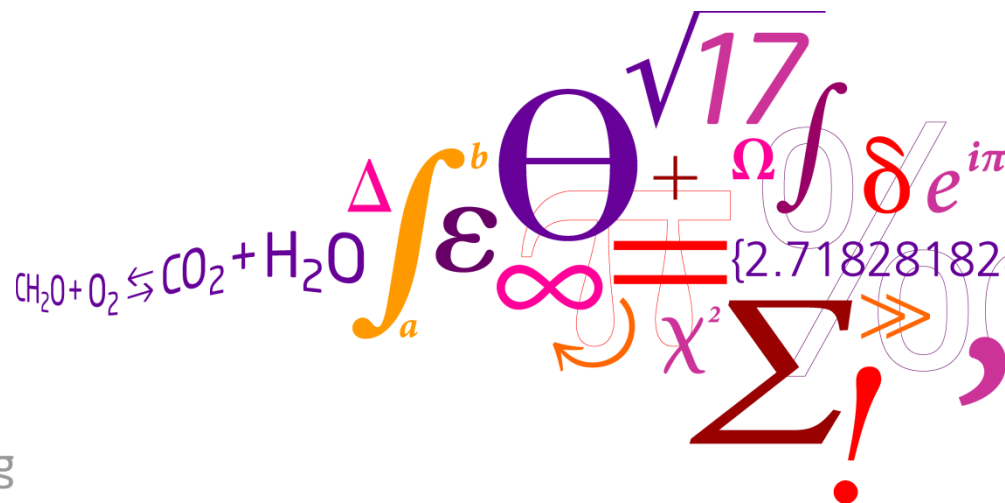
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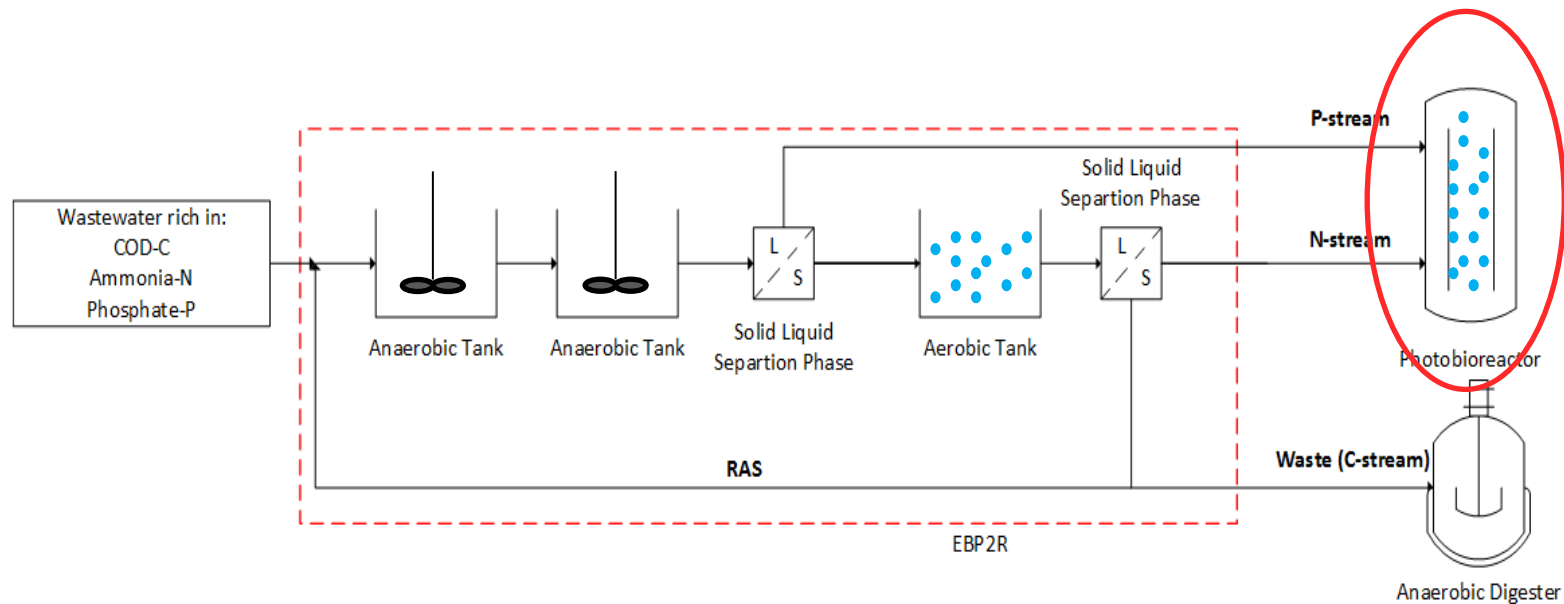
# Modeling green microalgal growth, nutrient uptake and storage in the ASM framework

Dorottya S. Wágner, **Borja Valverde-Pérez**, Mariann Sæbø, Marta Bregua de la Sotilla, Jonathan van Wageningen, Barth F. Smets, Benedek Gy. Plósz



# Motivation

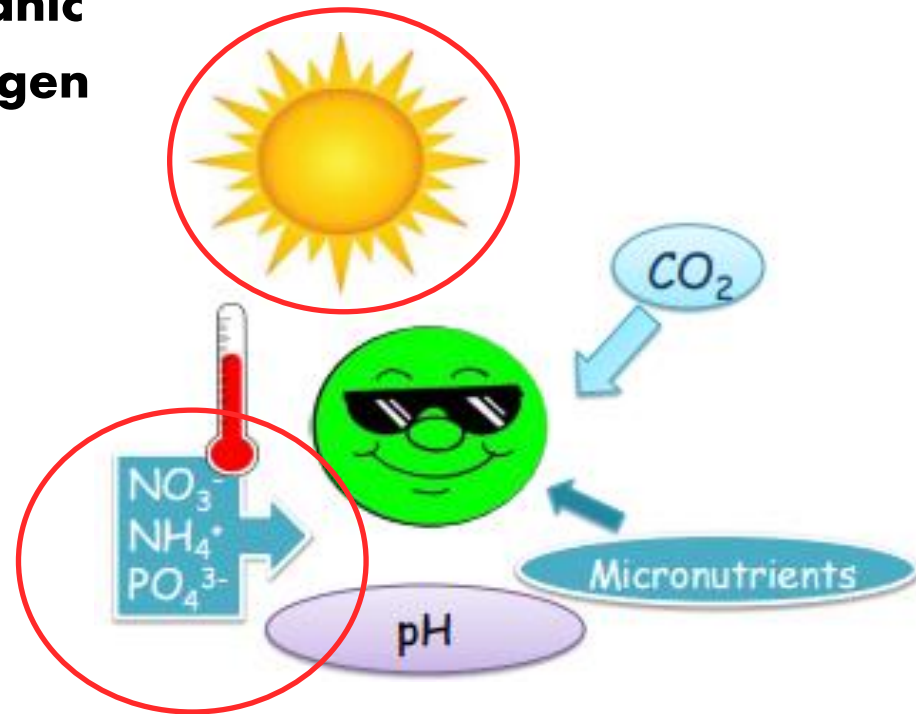
- **Microalgae photobioreactors can be used for wastewater treatment as:**
  - **Tertiary treatment step for nutrient removal**
  - **Nutrient recovery technology due to the phosphorus and nitrogen internal storage**



Valverde-Pérez et al. (2015)

# Motivation

- Available models are good, however the applicability may be limited:
  - By the number of considered variables
  - By the model structure
- Factors affecting microalgal growth:
  - Carbon, both organic and inorganic
  - Nutrients: phosphorus and nitrogen
  - pH
  - Light
  - Micronutrients (e.g. iron)



# Agenda

- **Model development**
- **Targeted experiments for parameter estimation:**
  - **Green microalgae identification and equipment**
  - **Microbatch and 1-L batch experiments**
  - **Open pond experiments**
- **Model evaluation**

# Model development (ASM-A)

- **Mechanistic** description of **biokinetic** processes: multiple-substrate kinetic process rate equations based on literature

Model	Autotrophic algal growth										Heterotrophic algal growth
	Nutrients		DIC	Light							
	N	P		Monod	Haldane	Steele	P&E	P&J	Smith	Poisson	
Moya et al. <sup>46</sup>											H
Bouterfas et al. <sup>48</sup>											
Ambrose et al. <sup>31</sup>	D	D									
Wolf et al. <sup>32</sup>	M										
Bougaran et al. <sup>42</sup>	D	D									
Quinn et al. <sup>33</sup>	D										
Broekhuizen et al. <sup>34</sup>	M										
Skjelbred et al. <sup>49</sup>											
Guest et al. <sup>35</sup>	D										
Decostere et al. <sup>36</sup>											
Coppens et al. <sup>38</sup>	M										
Van Wageningen et al. <sup>52</sup>											
ASM-A (This study)	D										M

D: Droop

M: Monod

H: Haldane

# Model development (ASM-A)

- Developed as an **extension of ASM-2d**, so compatible with activated sludge models
- Units and nomenclature expressed according to the **ASM** (activated sludge modelling) **framework**

Component	NH <sub>4</sub>	NO <sub>3</sub>	Internal quota N	PO <sub>4</sub>	Internal quota P	Inorg. carbon	Acetate	O <sub>2</sub>	Algal Biomass	Inert Particulates
Symbol	S <sub>NH4</sub>	S <sub>NO</sub>	X <sub>Alg,N</sub>	S <sub>PO4</sub>	X <sub>Alg,PP</sub>	S <sub>Alk</sub>	S <sub>A</sub>	S <sub>O2</sub>	X <sub>Alg</sub>	X <sub>I</sub>
Unit	gN/m <sup>3</sup>	gN/m <sup>3</sup>	gN/m <sup>3</sup>	gP/m <sup>3</sup>	gP/m <sup>3</sup>	gC/m <sup>3</sup>	gCOD/m <sup>3</sup>	gCOD/m <sup>3</sup>	gCOD/m <sup>3</sup>	gCOD/m <sup>3</sup>
Stoichiometric Matrix										
Uptake and storage of nitrogen from NH <sub>4</sub>	-1		1							
Uptake and storage of nitrogen from NO <sub>3</sub>		-1	1							
Uptake and Storage of PO <sub>4</sub>				-1	1					
Autotrophic growth			-i <sub>NXalg</sub>		-i <sub>PXalg</sub>	-1/Y <sub>Xalg,SAlk</sub>		1/(2.67*Y <sub>Xalg,SAlk</sub> )	1	
Heterotrophic growth			-i <sub>NXalg</sub>		-i <sub>PXalg</sub>	1/(0.4*Y <sub>Xalg,SA</sub> )	-1/(1.067*Y <sub>Xalg,SA</sub> )	-1/(1.067*Y <sub>Xalg,SA</sub> )	1	
Decay	i <sub>NXalg</sub> -f <sub>Xi</sub> *i <sub>NXalg,D</sub>			i <sub>PXalg</sub> -f <sub>Xi</sub> *i <sub>PXalg,D</sub>				-(1-f <sub>Xi</sub> )	-1	f <sub>Xi</sub>

# Microalgal nutrient uptake and storage

- Processes 1&2: uptake and storage of nitrogen using ammonia and nitrate as nitrogen source

$$k_{NH_4} \cdot \frac{S_{NH_4}}{S_{NH_4} + K_{NH_4,Alg}} \cdot \frac{X_{Alg,Nmax} \cdot X_{Alg} - X_{Alg,N}}{X_{Alg,Nmax} \cdot X_{Alg}} \cdot X_{Alg}$$

$$k_{NO} \cdot \frac{S_{NO}}{S_{NO} + K_{NO,Alg}} \cdot \frac{K_{NH_4,Alg}}{K_{NH_4,Alg} + S_{NH_4}} \cdot \frac{X_{Alg,Nmax} \cdot X_{Alg} - X_{Alg,N}}{X_{Alg,Nmax} \cdot X_{Alg}} \cdot X_{Alg}$$

- Process 3: uptake and storage of phosphate

$$k_{PO_4} \cdot \frac{S_{PO_4}}{S_{PO_4} + K_{PO_4,Alg}} \cdot \frac{X_{Alg,PPmax} \cdot X_{Alg} - X_{Alg,PP}}{X_{Alg,PPmax} \cdot X_{Alg}} \cdot X_{Alg}$$



# Microalgal growth and decay

## ● Processes 4: autotrophic growth rate

$$\mu_{A,max} \cdot \left(1 - \frac{X_{Alg,Nmin} X_{Alg}}{X_{Alg,N}}\right) \cdot \left(1 - \frac{X_{Alg,PPmin} X_{Alg}}{X_{Alg,PP}}\right) \cdot \frac{S_{Alk}}{S_{Alk} + K_{Alk}} \cdot \frac{I_{Av}}{I_S} \cdot e^{1 - \frac{I_{Av}}{I_S}} \cdot X_{Alg}$$

## ● Process 5: heterotrophic growth rate

$$\mu_{H,max} \cdot \left(1 - \frac{X_{Alg,Nmin} X_{Alg}}{X_{Alg,N}}\right) \cdot \left(1 - \frac{X_{Alg,PPmin} X_{Alg}}{X_{Alg,PP}}\right) \cdot \frac{S_A}{S_A + K_A} \cdot \frac{S_{O2}}{S_{O2} + K_{O2}} \cdot \frac{K_I}{K_I + I_{Av}} \cdot X_{Alg}$$

## ● Process 6: decay

$$b_{Xalg} \cdot X_{Alg}$$

# Agenda

- **Model development**

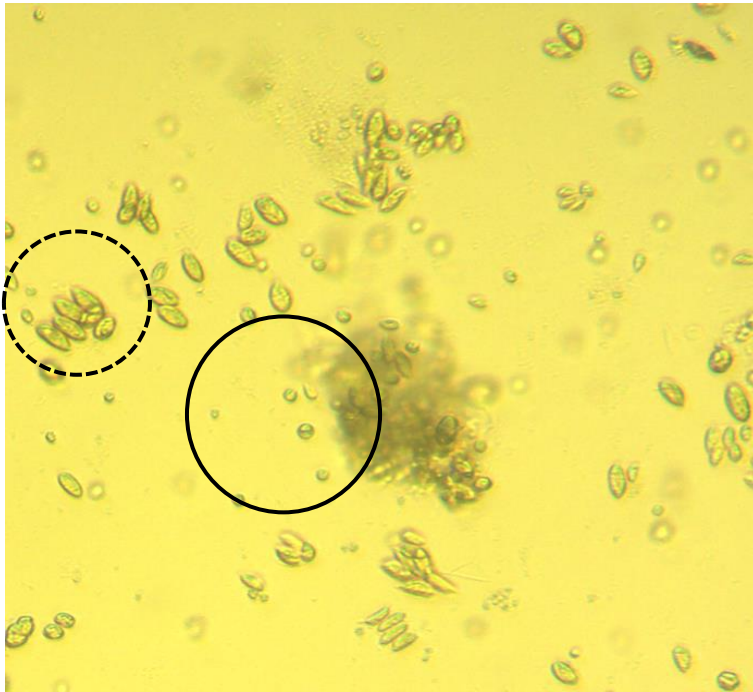
- **Targeted experiments for parameter estimation:**

- **Green microalgae identification and equipment**
- **Microbatch and 1-L batch experiments**
- **Open pond experiments**

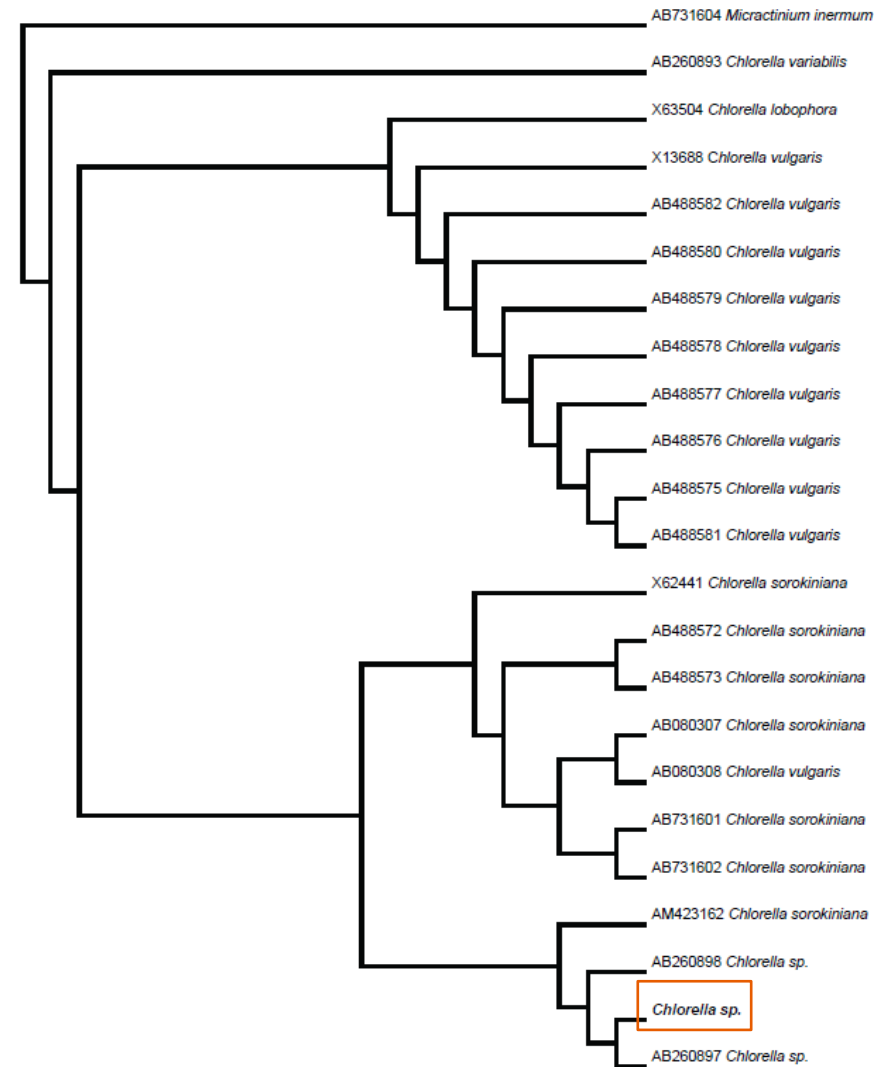
- **Model evaluation**

# Microalgal culture

- Mixed green microalgal culture consists mainly of ***Chlorella sp.*** (*C. sorokiniana*) and ***Scenedesmus sp.***



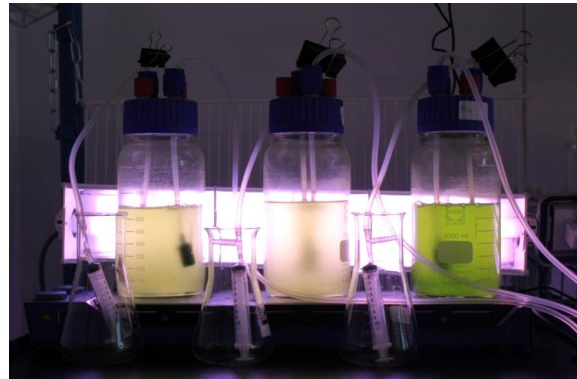
Microscope image of the mixed green microalgae culture in the batch experiment.



# Reactors

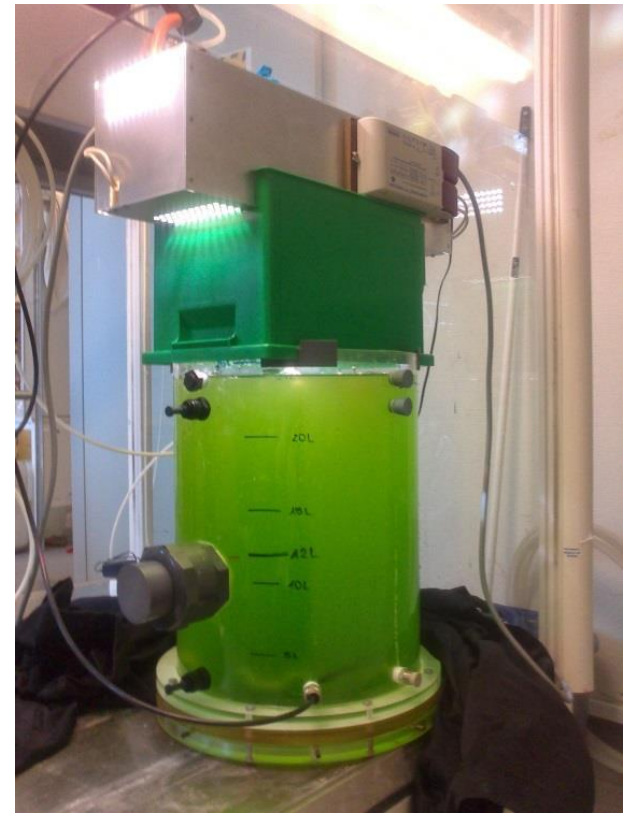


2 mL microbatches



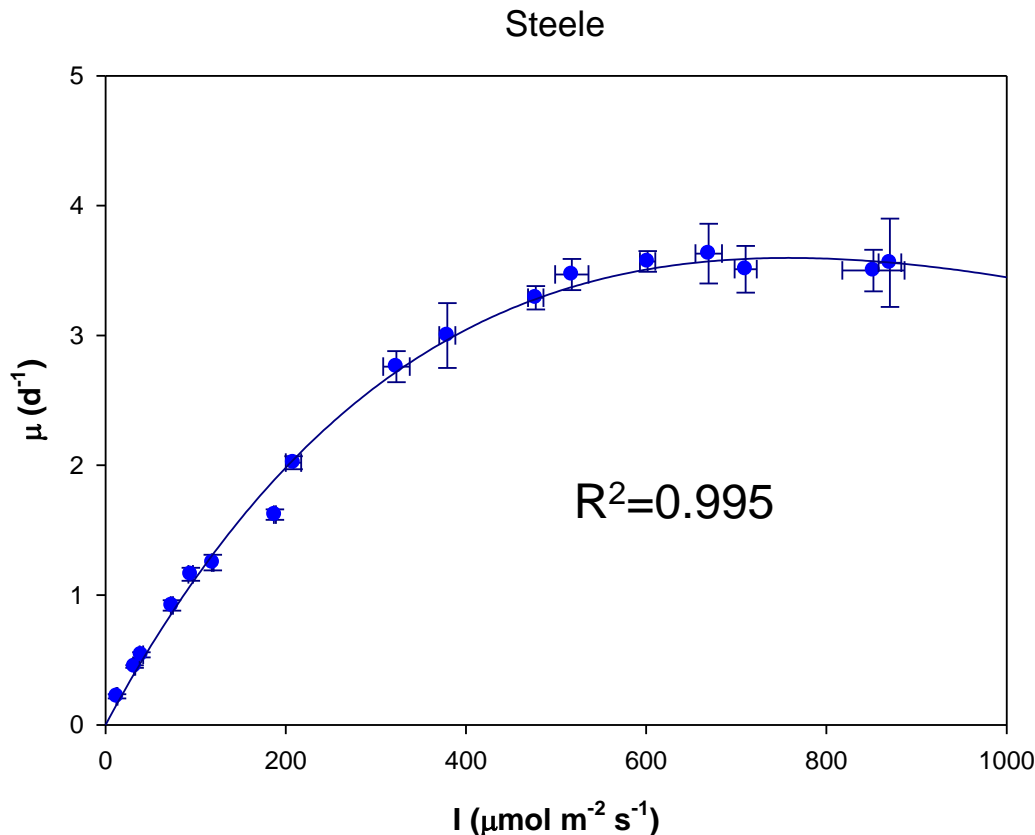
1-L batches

24 L open airlift PhBR



# Microbath experiments: light intensity effect

## Assessing the specific growth rate under different light intensities

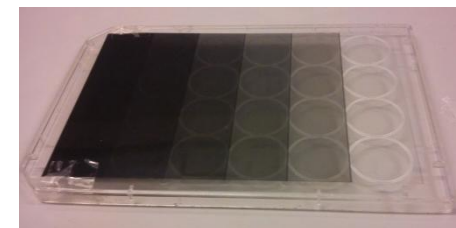


Steele equation:

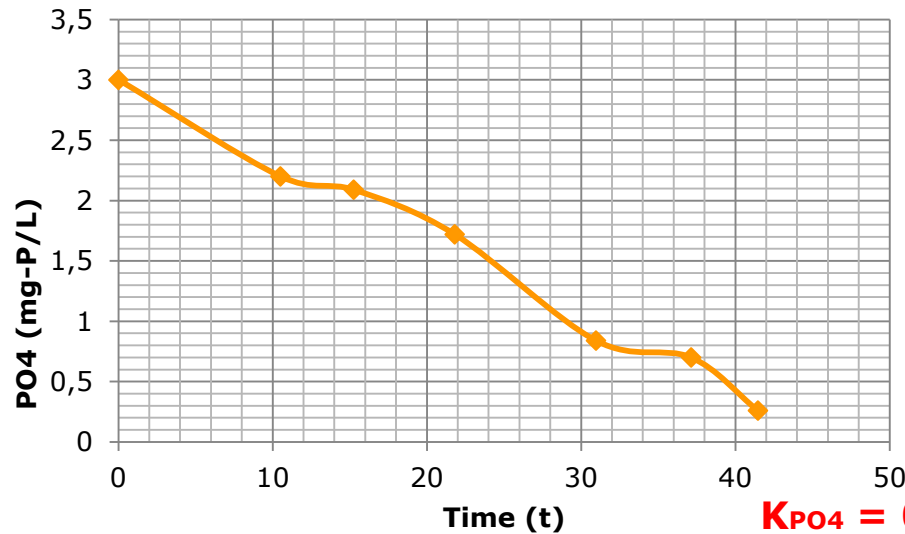
$$\mu = \frac{\mu_{max} * I}{I_s} \exp^{1-I/I_s}$$

$$\mu_{max} = 3.6 \text{ d}^{-1}$$

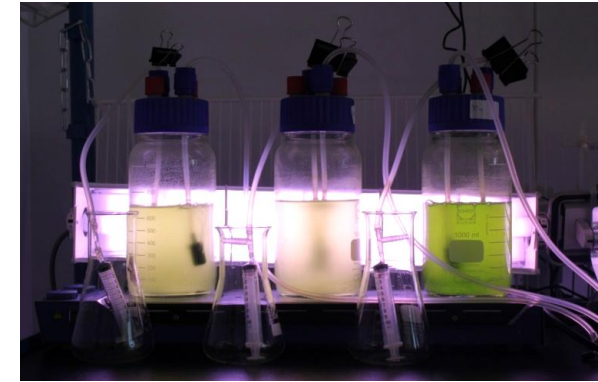
$$I_s = 758 \text{ μmol m}^{-2} \text{ s}^{-1}$$



# Nutrient uptake and storage: P-uptake and storage



Parameter  
estimation  
using Simplex

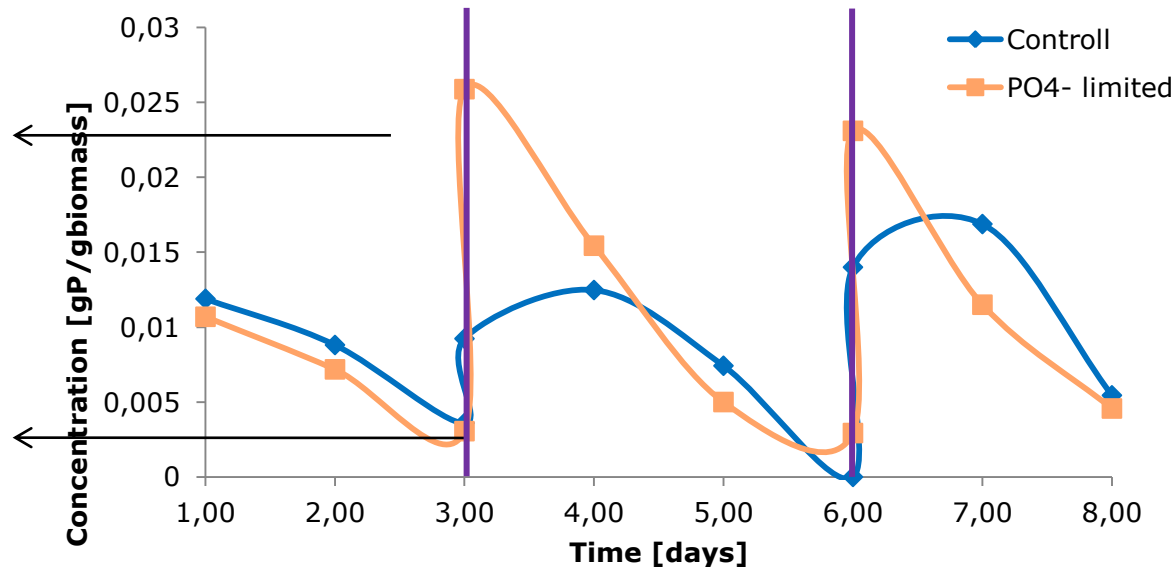


$$I_{av} = 100 \mu\text{mol m}^{-2}\text{s}^{-1}$$

$$K_{PO4} = 0.71 \text{ mgPO}_4\text{-P/L}$$

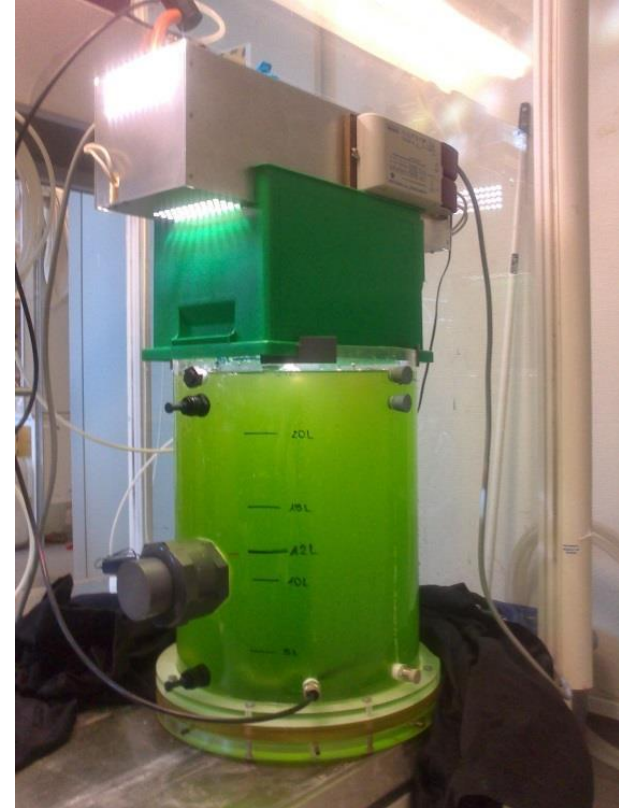
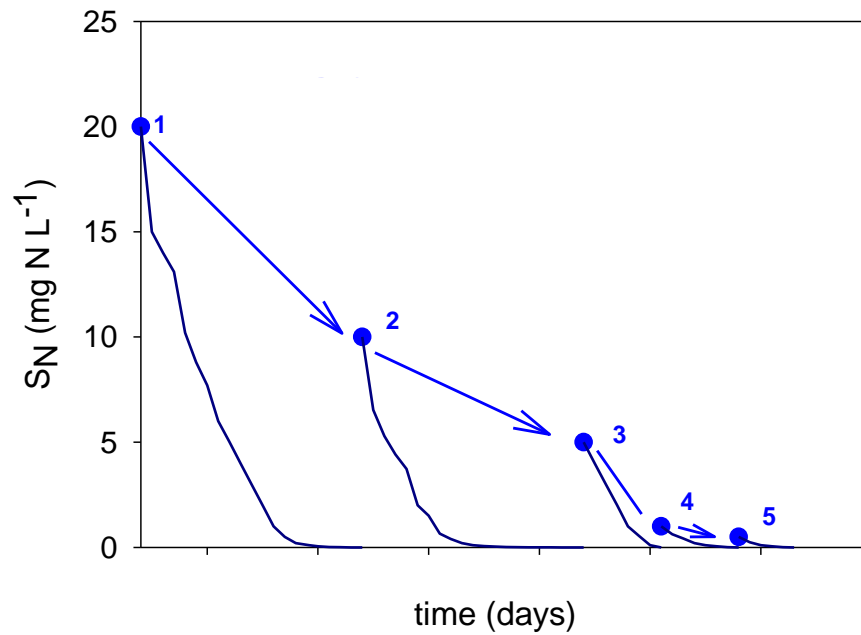
$$X_{Alg, PP_{max}} = 0.025 \text{ gP/gBiomass}$$

$$X_{Alg, PP_{min}} = 0.002 \text{ gP/gBiomass}$$



# N-uptake and storage

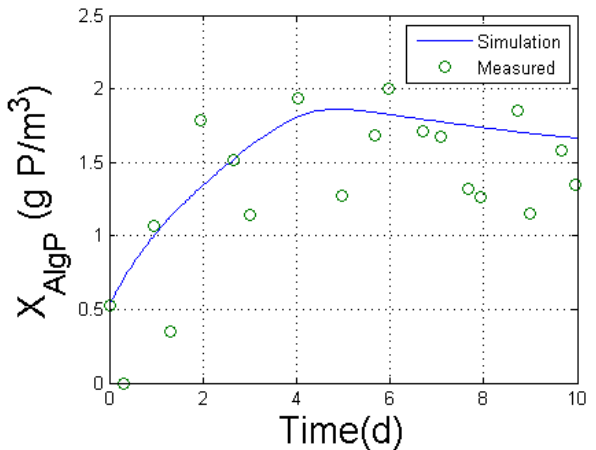
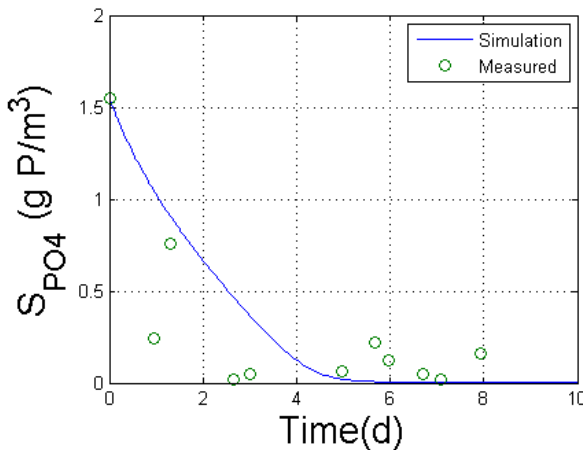
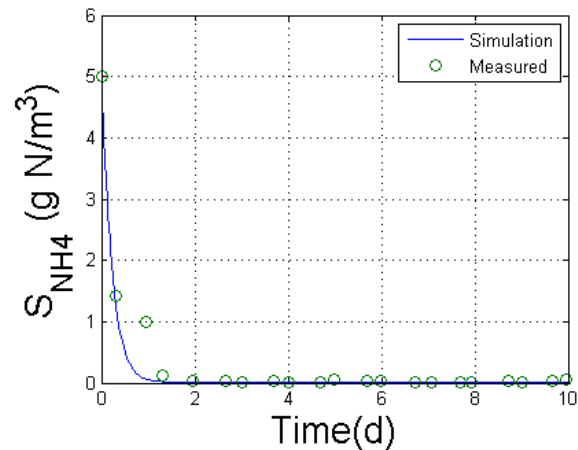
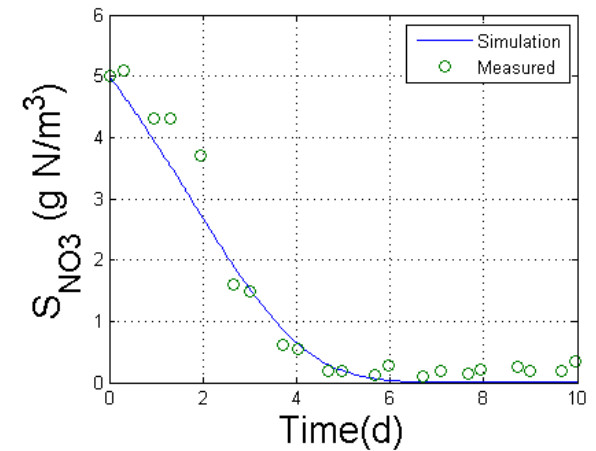
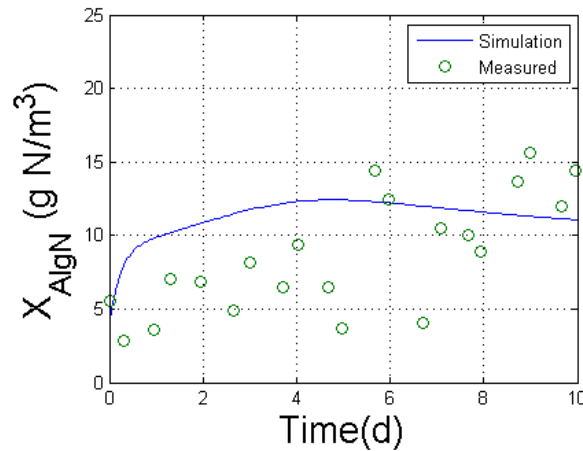
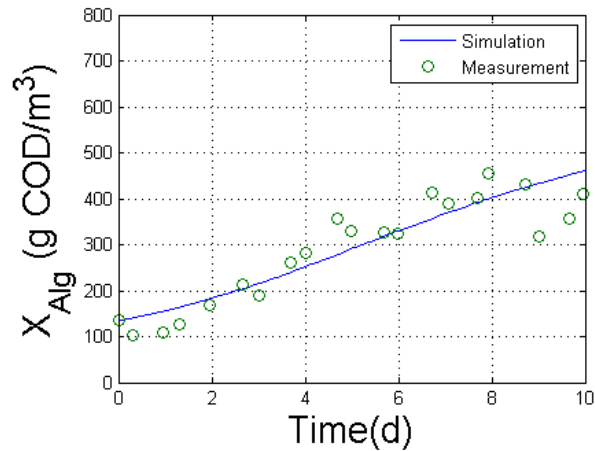
Cycle	Initial N conc. (g N/m <sup>3</sup> )
1	20
2	10
3	5
4	1
5	0.5



**Parameter  
estimation using  
Simplex**

# ASM-A calibration

## Model calibration using data from cycle 2





# Parameter Values

Parameter	Microplate	1-L batch	24-L batch	Literature Value	Unit
$\mu_{A,max}$	$3.6 \pm 0.04^a$	$3.3 \pm 0.55^e$	<b>4.19±1.19<sup>g</sup></b>	$4.7^{30}; 0.1-11^{36}; 1.4^{70}; 0.15^{71}; 2.64^{67}; 1.47^{67}; 3.26^{72}$	$d^{-1}$
$\mu_{H,max}$	$0.75 \pm 0.1^b$	<b><math>3.23 \pm 1.1^f</math></b>	-	$14^{37}; 3.48^{73}; 2.35-2.64^{67}; 0.96-1^{67}$	$d^{-1}$
$I_s$	<b><math>758.2 \pm 22.9^a</math></b>	-	-	-	$\mu mol\ m^{-2}\ s^{-1}$
$X_{Alg,PPmin}$	-	<b><math>0.002\pm0.0008^e</math></b>	-	-	$g\ P\cdot g^{-1}COD$
$X_{Alg,Nmin}$	-	<b><math>0.014\pm0.0015^e</math></b>	-	$0.023^{37}; 0.02^{72}$	$g\ N\cdot g^{-1}COD$
$X_{Alg,PPmax}$	-	<b><math>0.02\pm0.008^e</math></b>	-	-	$g\ P\cdot g^{-1}COD$
$X_{Alg,Nmax}$	-	<b><math>0.9\pm0.5^e</math></b>	-	$0.0683^{37}; 0.067^{72}$	$g\ N\cdot g^{-1}COD$
$K_{NO,Alg}$	-	$3.39 \pm 1.89^e$	<b><math>8.22\pm0.97^g</math></b>	$0.0602^{37}; 0.3^{71}$	$g\ N\cdot m^{-3}$
$K_{NH4,Alg}$	-	$2.4 \pm 0.42^e$	<b><math>3.91\pm0.88^g</math></b>	$5^{34}; 0.1-31.5^{71}$	$g\ N\cdot m^{-3}$
$K_{PO4,Alg}$	-	$0.25 \pm 0.18^e$	<b><math>0.36\pm0.046^g</math></b>	$0.046-10.5^{71}$	$g\ P\cdot m^{-3}$
$k_{NH4,Alg}$	-	$2.97 \pm 2.42$	<b><math>0.22\pm0.01^g</math></b>	-	$g\ N\cdot g^{-1}COD\cdot d^{-1}$
$k_{NO,Alg}$	-	$2.97 \pm 2.42$	<b><math>0.018\pm0.012^g</math></b>	$0.49^{37}; 0.43^{72}$	$g\ N\cdot g^{-1}COD\cdot d^{-1}$
$k_{PO4,Alg}$	-	$0.066 \pm 0.042$	<b><math>0.028\pm0.021^g</math></b>	-	$g\ P\cdot g^{-1}COD\cdot d^{-1}$
$K_{Alk}$	-	-	-	<b><math>3^{34}; 0.035-0.29^{71}</math></b>	$g\ C\cdot m^{-3}$
$K_A$	$11.87 \pm 5.2^b$	<b><math>89 \pm 28^f</math></b>	-	-	$gCOD\cdot m^{-3}$
$K_{i,A}$	$550.58 \pm 232.1^b$	-	-	-	$gCOD\cdot m^{-3}$
$K_I$	<b><math>878.6\pm75^d</math></b>	-	-	-	$\mu mol\ m^{-2}s^{-1}$
$K_O$				$2\ (20\% \text{ of } DO_{sat})^{56}$	$gCOD\ m^{-3}$
$b_{Xalg}$	-	-	<b><math>0.091\pm0.0^g</math></b>	$0.072\ (2\% \cdot \mu_{A,max})^{33}; 3.7\% \mu_{A,max}^{30}; 0.003-0.1^{71}$	$d^{-1}$
$Y_{Alk}$	-	-	-	<b><math>2.33; 1.06^{71}</math></b>	$g\ COD\cdot g^{-1}C$
$Y_{Ac}$	-	<b><math>0.39^f</math></b>	-	$0.34^{45}$	$g\ COD\cdot g^{-1}COD$
$iP_{Xalg}$	-	-		<b><math>0.00067^{31,34}</math></b>	$g\ P\cdot g^{-1}COD$
$iN_{Xalg}$	-	-		<b><math>0.0049^{31}; 0.0534^{34}</math></b>	$g\ N\cdot g^{-1}COD$
$fX_I$	-	-		<b><math>0.1</math></b>	$g\ COD\cdot g^{-1}COD$
$N_{XAlgD}$				<b><math>0.00441</math></b>	$g\ N\cdot g^{-1}COD$
$P_{XAlgD}$				<b><math>0.000603</math></b>	$g\ P\cdot g^{-1}COD$

# Agenda

- **Model development**
- **Targeted experiments for parameter estimation:**
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  - **Microbatch and 1-L batch experiments**
  - **Open pond experiments**
- **Model evaluation**

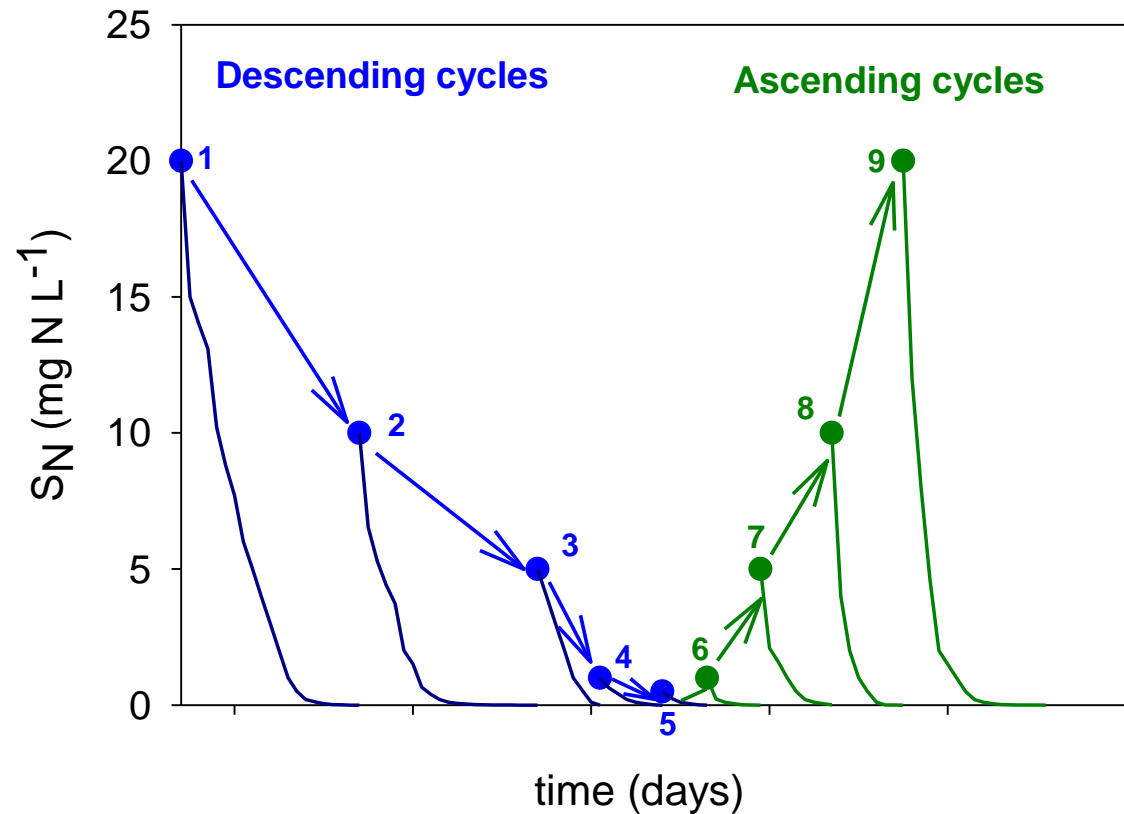
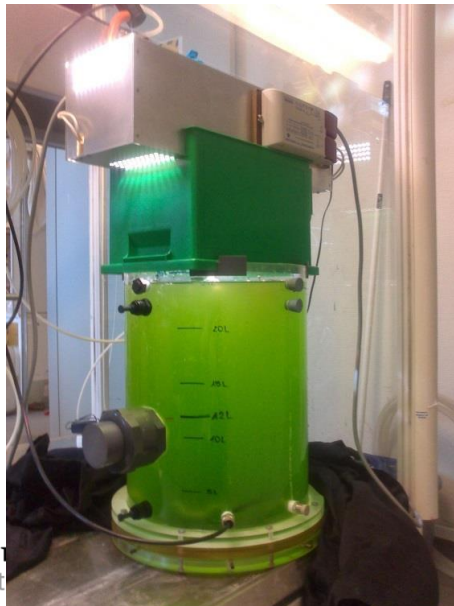
# Model evaluation: assessment of parameter variability impact

## Research questions:

- What is the influence of culture history and/or substrate availability on parameter estimates?
- Can we use a default parameter set?
- Can we explain the discrepancy as a result of parameter variability?

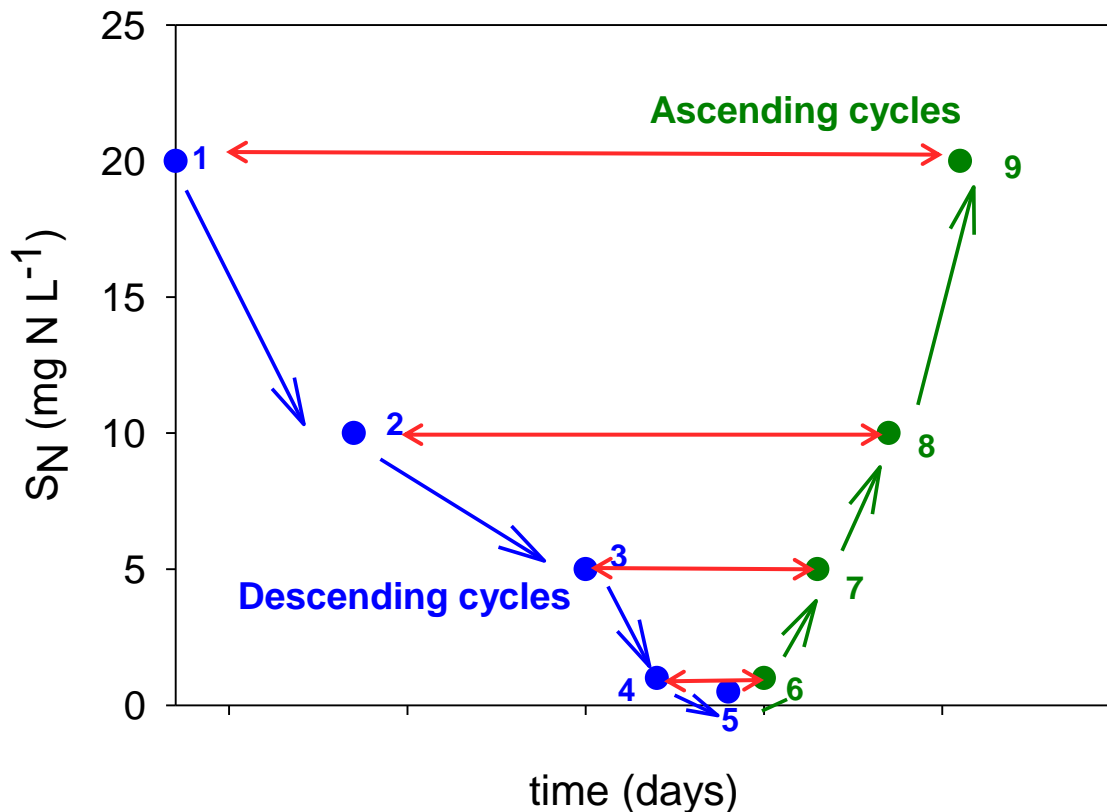
# Model evaluation: experimental design

Cycle	Initial N conc. (g N/m <sup>3</sup> )
1 and 9	20
2 and 8	10
3 and 7	5
4 and 6	1
5	0.5



# Model evaluation: two evaluation steps

- Does culture history affect parameter values?
  - Parameter sets obtained through the descending cycles confronted with data from ascending cycles
  - Janus coefficient



# Model evaluation: two evaluation steps

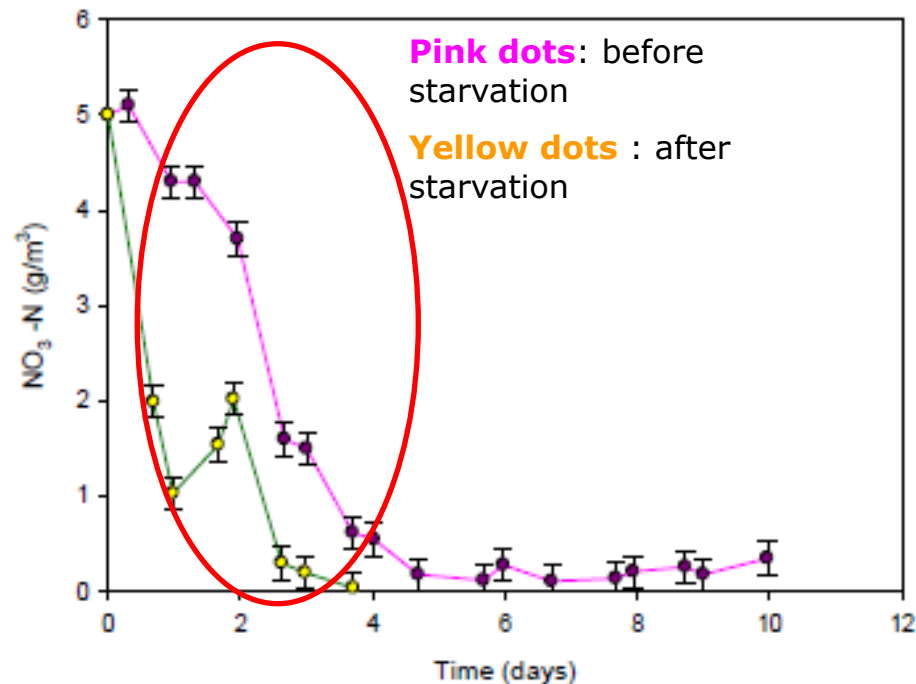
Cycle 2-8	RMSE calibration	RMSE evaluation	Janus coefficient
Ammonium in bulk liquid ( $S_{NH4}$ )	0.72	0.44	0.61
Nitrate in bulk liquid ( $S_{NO3}$ )	0.71	14.00	19.72
Phosphate in bulk liquid ( $S_{PO4}$ )	0.91	0.51	0.56
Algal biomass ( $X_{Alg}$ )	0.19	0.1	0.53
Nitrogen quota ( $X_{AlgN}$ )	1.27	0.70	0.55
Phosphorous quota ( $X_{AlgP}$ )	0.91	0.14	0.15
Total	4.71	15.9	3.38

## Nitrate prediction in the other cycles:

Cycle	RMSE calibration	RMSE evaluation	Janus coefficient
1-9	0.24	23.9	99.58
3-7	0.26	3.85	14.81
4-6	0.23	9.35	40.65

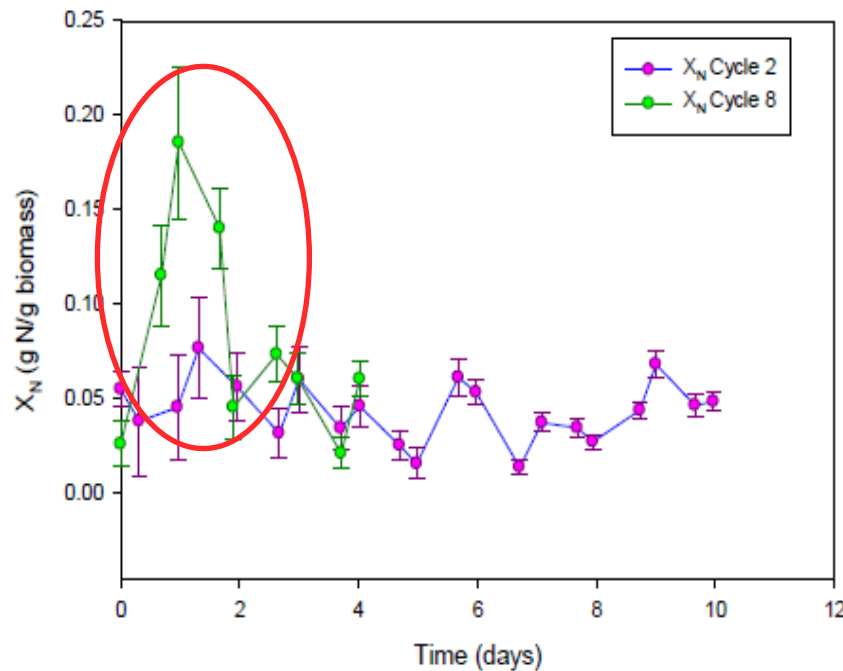
- $J \sim 1$  calibrated model prediction is good
- $J \gg 1$  calibrated model prediction fails

# Model evaluation: experimental results



- The **nitrate uptake** after starvation conditions is **enhanced**

# Model evaluation: experimental results



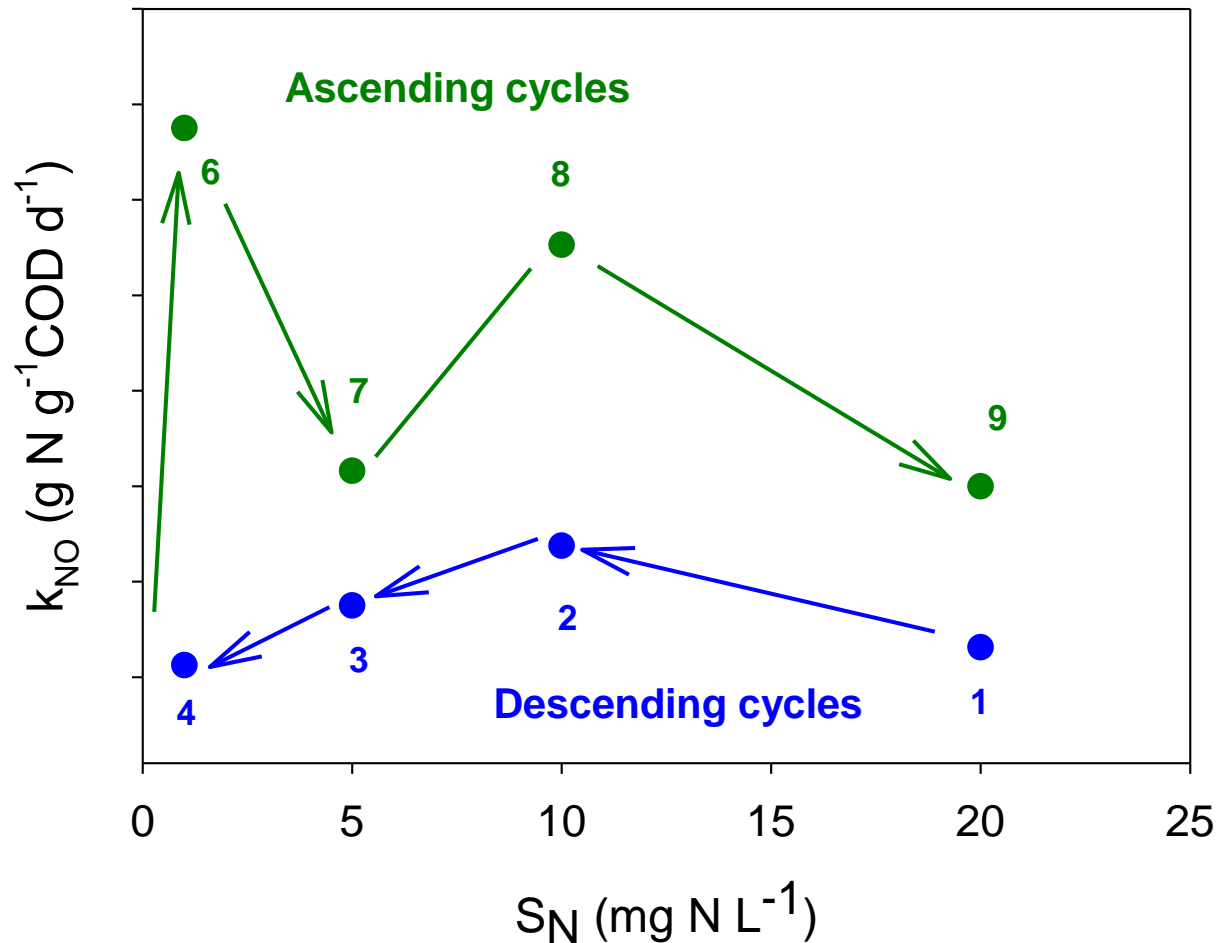
**Pink dots:** before starvation

**Green dots:** after starvation

- After the **N quota** is replenished there is a **temporary enhanced N storage**

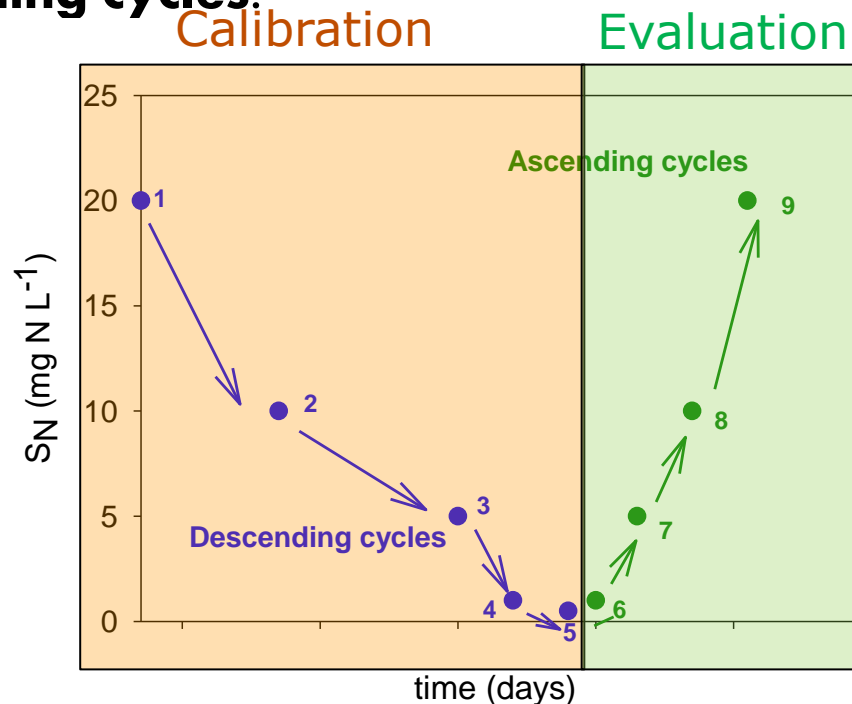


# Model evaluation: parameter variability



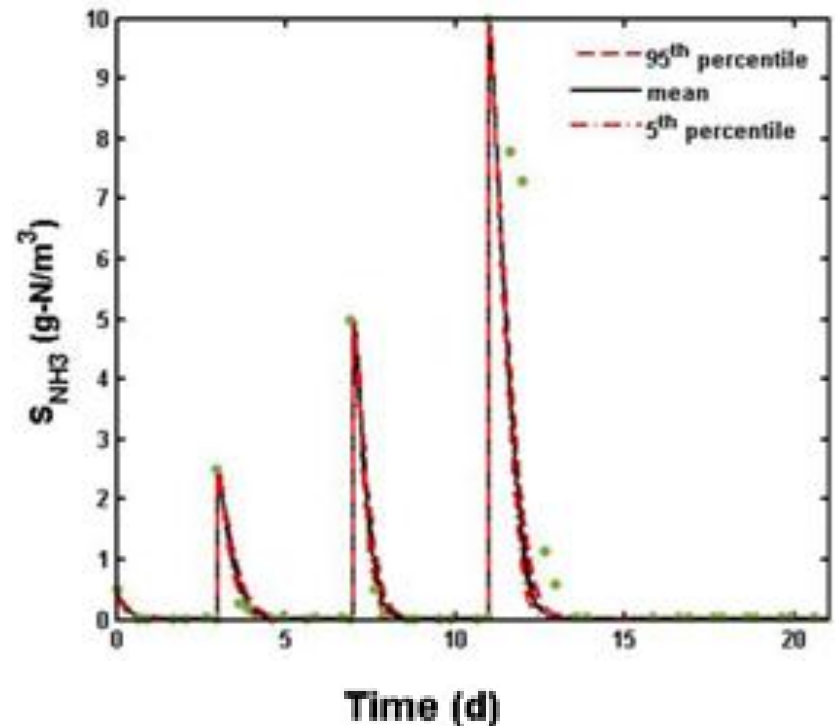
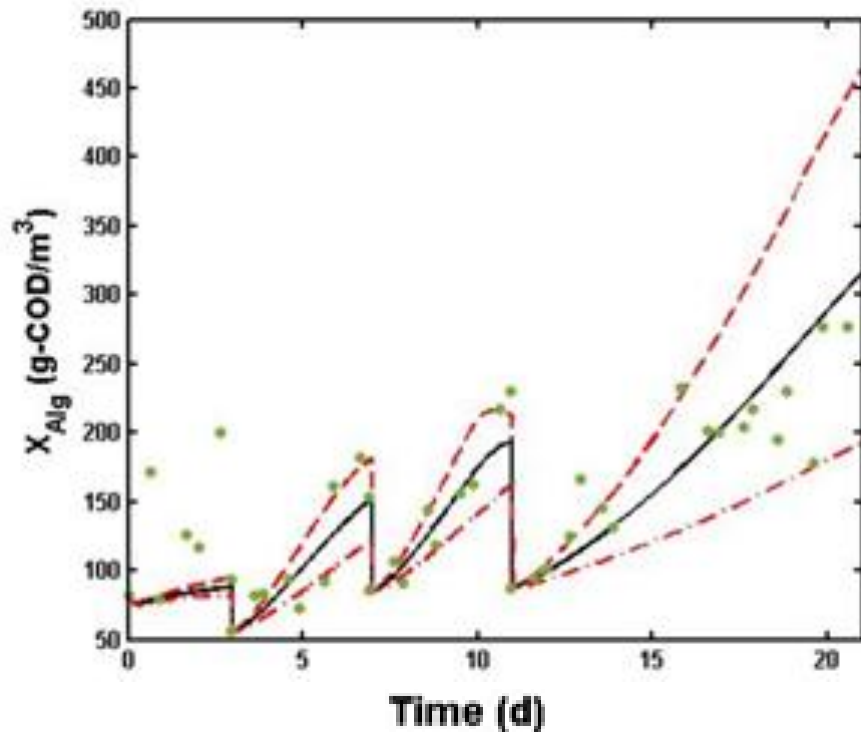
# Model evaluation: two evaluation steps

- Can we use an average parameter set? Can we explain the discrepancy as a result of parameter variability?
- Monte Carlo simulations run on the 4 ascending cycles
- Parameter values: mean values of the estimated parameters through descending cycles.
- Probability range: standard deviation of the mean values through descending cycles.



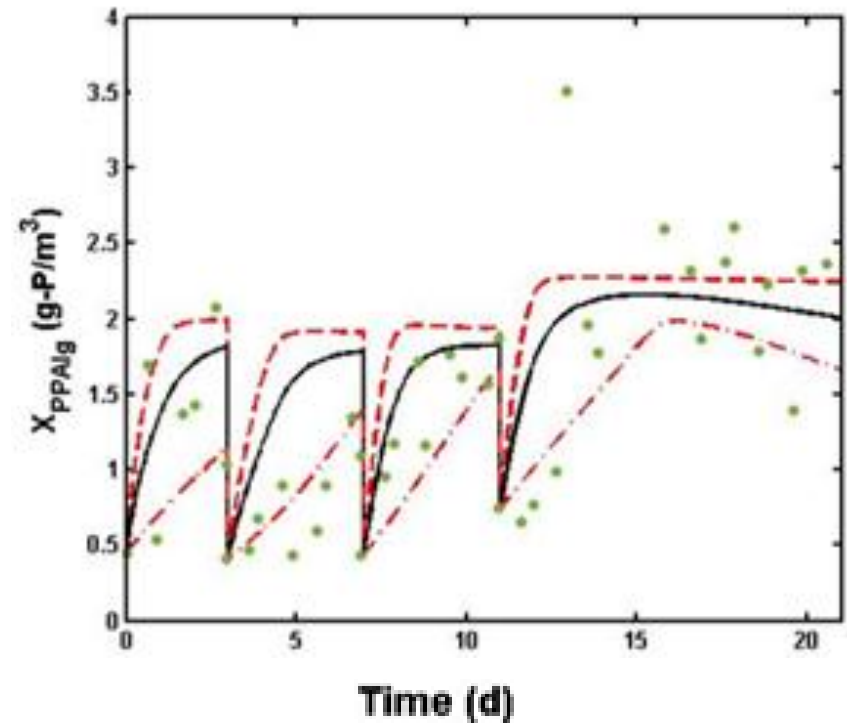
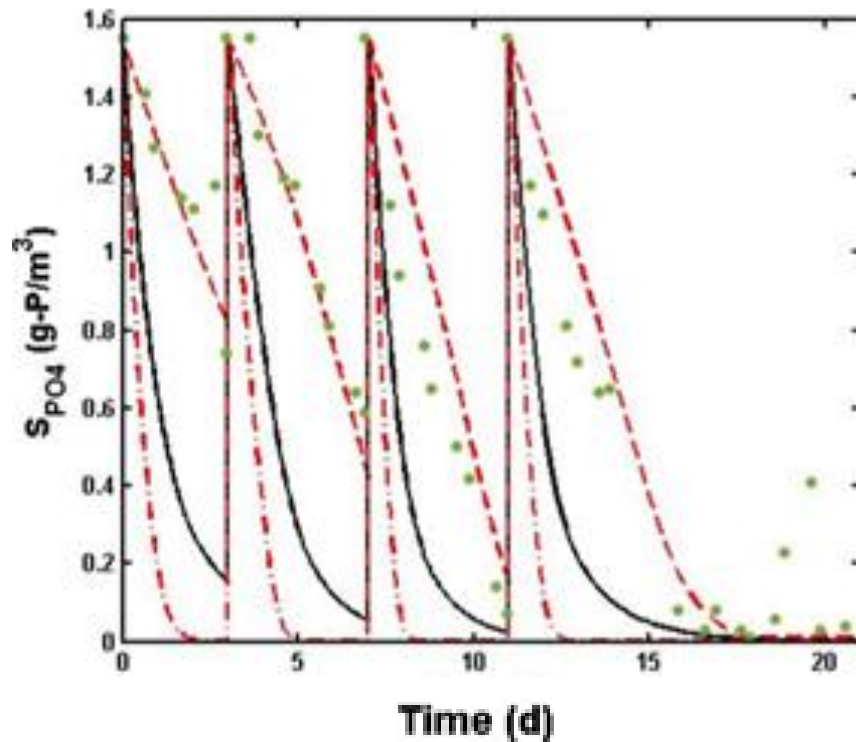
# Model evaluation: second steps

- **Algal biomass & ammonia: discrepancies can be explained by parameter variability**



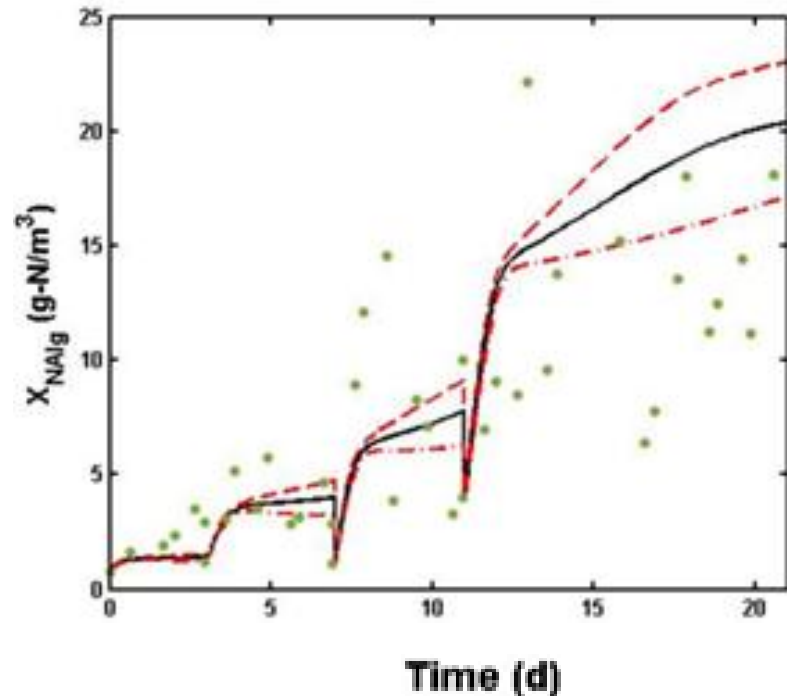
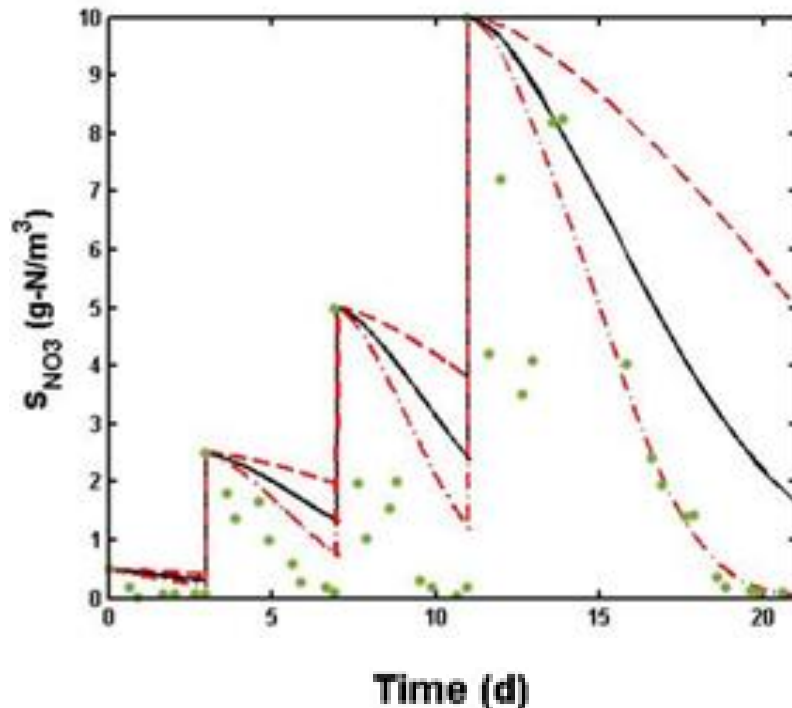
# Model evaluation: two evaluation steps

- Soluble and stored phosphate: discrepancies can be explained by parameter variability



# Model evaluation: two evaluation steps

- Soluble nitrate & stored nitrogen: the model prediction is compromised by
  - Culture history for nitrate
  - Substrate availability for nitrogen storage



# Concluding remarks

- A novel process model in the ASM framework for predicting algal behavior in PBR has been identified, calibrated and critically evaluated
- Different scale lab experiments have been used to estimate different parameter sets.
- The model can predict algal biomass, ammonia, phosphate and internal PP quota using a mean parameter set
- Maximum nitrate uptake rate depends on the history of the culture

## Future perspectives

- Model extensions including physic-chemical processes:
    - Mass transfer
    - pH
    - Light attenuation
    - Hydrodynamics
  - Model extensions relevant to other end-uses: lipid accumulation for biodiesel
  - Model calibration and evaluation using other microalgae species
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# Aknowlegement



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## Thank you for your attention!